J. Health Biol Sci. 2022;10(1):1-5 doi: 10.12662/2317-3206jhbs.v10i1.4490.p1-5.2022

ORIGINAL ARTICLE

Effects of *Astragalus membranaceus, Peumus boldus* and *Curcuma longa* extracts in diabetic rats

Efeitos de extratos de *Astragalus membranaceus, Peumus boldus* e *Curcuma longa* em ratos com diabetes induzida

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Abstract

Objectives: the aim of this study was to evaluate the effects of the association of dry extracts of *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* in rats with induced diabetes. **Methods**: After the induction of type 2 diabetes by intraperitoneal streptozotocin, male Wistar rats were randomly assigned to groups (n=6) and treated for 20 days. The extracts were suspended in water and administered through orogastric gavage once daily as described: Group I: healthy control (saline); group II: received *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* (400 mg/kg/day of each dry extract); group III: received *Astragalus* membranaceus, *Peumus boldus*, *Curcuma longa* (400 mg/kg/day of each dry extract) and glibenclamide (15 mg/kg/day). Fasting glucose, glucose tolerance, alanine aminotransferase, aspartate aminotransferase and fructosamine were evaluated. **Results**: Fasting blood glucose and glucose tolerance for groups II and III were influenced by treatments ($p<0.05$). The extracts did not significantly influence the efficacy of glibenclamide. **Conclusion**: The results found in this study allow us to consider that it is not possible to conclude that the compounds evaluated are not effective in DM in rats, due to variables such as total treatment period, doses, size of pancreatic injury caused by streptozotocin, and diet profile may have influenced the results. The studied compounds have potential for application in diabetes and further studies should be carried out to adjust the treatment.

Keywords: AST; ALT; Diabetes mellitus; Streptozotocin; Blood glucose.

Resumo

Objetivos: avaliar os efeitos da associação de extratos secos de *Astragalus* membranaceus, *Peumus boldus* e *Curcuma longa* em ratos com diabetes induzida. **Métodos**: Após a indução de diabetes tipo 2 (DM) por estreptozotocina intraperitoneal, ratos Wistar machos foram distribuídos aleatoriamente em grupos (n=6) e tratados por 20 dias. Os extratos foram suspensos em água e administrados por gavagem orogástrica uma vez ao dia conforme descrito: Grupo I: controle saudável (solução salina); grupo II: recebeu *Astragalus* membranaceus, *Peumus boldus* e *Curcuma longa* (400 mg/kg/dia de cada extrato seco); grupo III: receberam *Astragalus* membranaceus, *Peumus boldus*, *Curcuma longa* (400 mg/kg/dia de cada extrato seco) e glibenclamida (15 mg/kg/dia). A glicemia de jejum, tolerância à glicose, alanina aminotransferase, aspartato aminotransferase e frutosamina foram avaliados. **Resultados**: A glicemia de jejum e a tolerância à glicose para os grupos II e III foram influenciadas pelos tratamentos ($p<0,05$). Os extratos não influenciaram significativamente na eficácia da glibenclamida. **Conclusão**: Os resultados encontrados neste estudo permitem considerar que não é possível concluir que os compostos avaliados não são eficazes no DM em ratos, devido às variáveis como tempo total de tratamento, doses e tamanho da lesão pancreática causada por estreptozotocina, além do perfil da dieta, que podem ter influenciado os resultados. Os compostos estudados têm potencial para aplicação em diabetes e mais estudos devem ser realizados para adequar o tratamento.

Palavras-chave: lAST; ALT; Diabetes mellitus; Estreptozocina; Glicemia.

INTRODUCTION

*Astragalus membranaceu*s (Radix Astragali) known as Huang Qi has ethnopharmacological relevance, as well as indications in Chinese medicine for insulin sensitivity, hepatic metabolism, intestinal glucose absorption, as well as antioxidant and anti-inflammatory effects[1]. The actions attributed to *A. membranaceus* are due to the presence of some polysaccharides, saponins, flavonoids and astragaloside, the latter used as a quality marker in some herbal medicines sold in Brazil[2].

Over the years, several authors have investigated its effects on the health of experimental animals, at different doses and routes. YI et al.[3] investigated the effect of daily intraperitoneal injections in mice (0.03 mL/10 g/day) being effective in preventing renal dysfunction. DUN et al.[4] studied the effect of *Astragalus* polysaccharides in oral treatment (200, 400 and 800 mg/kg/day) on the memory of Wistar rats with induced diabetes and found satisfactory results. HUANG et al.[5] demonstrated that the use of *Astragalus* polysaccharides prevented neuroinflammation and could act preventively in Alzheimer's disease, as well as an effect on increasing the sensitivity of adipocytes to insulin in cell culture with the "mouse 3T3-L1" strain with a dose-dependent effect. CHEN et al.[6] studied their effects on cardiomyopathies in mice based

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Conflict of interest: The authors declare that there is no conflict of interest.
Received: 2022 Jul 11; Revised: 2022 Dec 15; Accepted: 2022 Dec 20

on the knowledge that polysaccharides act on superoxide dismutase activity and H_2O_2 formation in animals with induced diabetes and demonstrated reduced production of free radicals and oxidative stress after 15 days of daily treatment. LIU et al.[7] studied the association of *Astragalus* saponins with curcumin finding effects such as reduction of proteinuria, urea, creatinine and protection of renal function. The systematic review by SALEHI et al.[8] describe *Astragalus membranaceus, Peumus boldus and Curcuma longa* as medicinal plants with potential for use in the control of DM.

Boldine ((S)-2,9-dihydroxy-1,10-dimethoxy-aporphine) is an alkaloid obtained from *Peumus boldus* and has been considered antioxidant, anti-inflammatory and antihyperglycemic [9].

The World Health Organization reports that the prevalence of type 2 diabetes mellitus has increased dramatically in countries of all socioeconomic levels, an estimated 422 million people have diabetes 10 and this disease is still incurable. Therefore, it is essential to search for affordable treatments. Considering that *A. membranaceus*, *Peumus boldus* and *Curcuma longa* have been reported in the literature as herbal medicines that have potential for application in the control of DM and there are no studies that associate them, we considered evaluating their association.

The aim of this study was to investigate the effects of *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* on the glycemic profile and liver health of rats with induced diabetes.

METHODS

Material

Standardized dry extracts of *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* (Florien Fitoativos™, Brazil), glibenclamide and strepotozocin (Cayman Chemical Company), Anasedan and Dopalen (Ceva™, Brazil), and enzyme assay kits (LaborLab™, Brazil). All other reagents used were of analytical grade and commercially obtained.

The extracts were suspended in water and administered through orogastric gavage once daily.

Ethical aspects

The experimental protocol performed in this study was approved by the Animal Research Ethics Committee, Pontifical Catholic University of Campinas (PUC Campinas) (Protocol number: 020/2019), according to the Guide for the Care and Use of Laboratory Animals. All applicable international, national, or institutional guidelines for the care and use of animals were followed.

Animals

Male non-isogenic albino Wistar rats (*Rattus norvegicus*) were obtained after weaning at the age of 30 days from the Multidisciplinary Center for Biological Investigation, Area of Laboratory Animal Science, of the Pontifical Catholic University of Campinas (PUC-Campinas). The animals were housed under appropriate light and ventilation conditions and received appropriate rat chow (NuvilabTM, Brazil), with free access to water.

Induction of Diabetes using STZ

Diabetes induction was performed according to a previous study, by intraperitoneal injection of streptozocin (STZ) in citrate buffer in fasting animals (70 mg/kg body weight), followed by gavage with 0.5 mL of 20% sucrose solution[11]. After 3 days, confirmation was performed by determining capillary glycemia in a blood sample obtained from the caudal vein by using a one touch glucometer (Accu-check®). Only the healthy control group (group I) did not receive the STZ injection.

Treatment and Sample collection

Animals were randomly divided into three groups (n=6) and treatments were performed by orogastric gavage once daily. Group I was treated with saline, Group II received *Astragalus membranaceus, Peumus boldus* and *Curcuma longa* (400 mg/kg/day of each dry extract) and Group III received *Astragalus membranaceus, Peumus boldus* and *Curcuma longa* (400 mg/day). kg/day of each) + glibenclamide (15 mg/kg/day). All animals received treatment for 20 days.

A blood sample was collected from the fasting animals for the determination of capillary blood glucose by using a one touch glucometer on the twentieth day of treatment (Accu-check®). Then, the last gavage was performed adding 20% sucrose in the formulations of each group and a new blood sample was collected after one hour for the determination of capillary blood glucose - glucose tolerance test.

The animals were anesthetized with Xylazine hydrochloride and Ketamine administered intramuscularly. Blood was collected by cardiac puncture for the determination of fructosamine, alanine-aminotransferase (ALT) and aspartate aminotransferase (AST), using LaborLab™ kits. After the above procedure, the animals were euthanized with excess anesthesia.

Statistical analysis

The statistical analyses were carried out using GraphPad Prism software, version 4.0 (GraphPad, Inc., San Diego, USA). All data were expressed as mean ± standard error of mean (S.E.M). Post hoc test of one-Way ANOVA was used to compare the differences of mean values between different groups. P values less than 0.05 were considered statistically significant.

RESULTS

Statistical analysis (Table 1 and Figure 1) indicated that the data

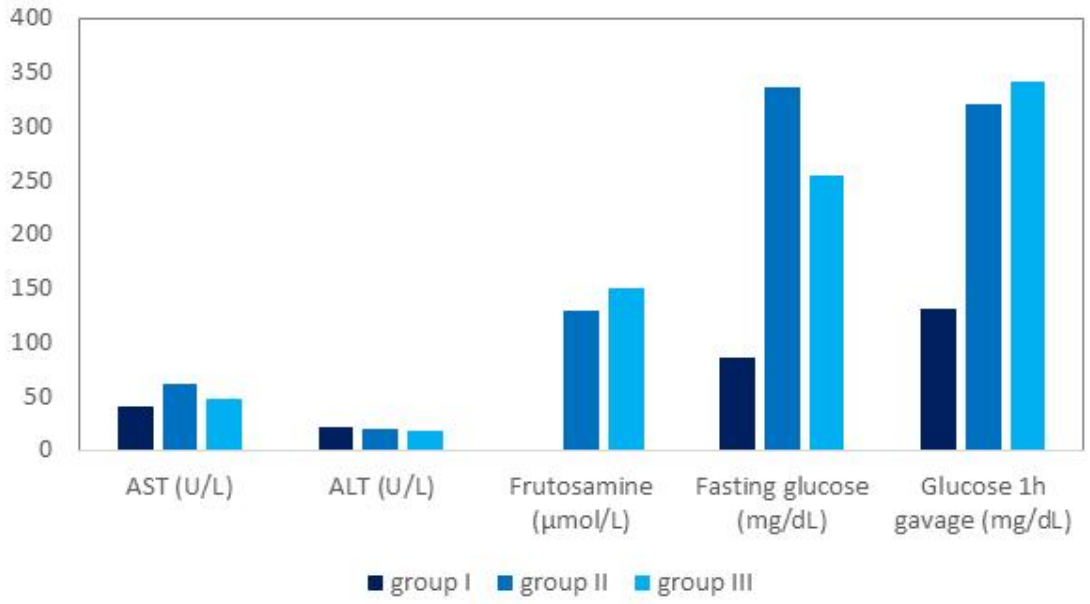
for groups II and III were different from those obtained for the control (group I) only for fasting glucose and glucose after 1h of the last gavage containing sucrose. The other parameters showed no difference between the groups.

Table 1. Alanine aminotransferase (ALT), aspartate amino transferase (AST), Fructosamine and blood glucose levels of experimental groups on day 20 of the treatment.

Results	Groups		
	I	II	III
AST (U/L)	40,57 ± 14	60,44 ± 34,80	47,73 ± 14,50
ALT (U/L)	21,05 ± 4,56	19,32 ± 7,52	17 ± 4,46
Fructosamine (µmol/L)	nd*	128,31 ± 29,10	149,49 ± 34,80
Fasting blood glucose (mg/dL)	85 ± 7,40*	336 ± 216,30	255 ± 80
Blood glucose (mg/dL) after 1h sucrose	130,67 ± 22,70*	321 ± 174,80	342,1 ± 131,30

Legend: Data are represented as mean ± SEM of six rats. Group I: healthy control; Group II: treated with *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* (400 mg/kg/day of each); Group III: treated with *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* (400 mg/kg/day of each) and glibenclamide (15 mg/kg/day); nd: not determined; *: p<0.05 for group I.

Figure 1. Alanine aminotransferase (ALT), aspartate amino transferase (AST), Fructosamine and blood glucose levels of experimental groups on day 20 of the treatment.



Regarding body weight, there was no variation between the groups and there was a gain of about 90 grams over the study period. We observed an increase in water consumption by groups II and III and a consequent increase in urine volume, but these were not quantified.

DISCUSSION

In the present study, the possible effects of standardized dry extracts in a streptozotocin (STZ) –induced diabetes model of rats were investigated. The same amount of 400 mg/kg was chosen for the administration of extracts of *A. membranaceus, Peumus boldus and Curcuma longa* in association or not with glibenclamide.

Statistical analysis indicated that the liver health of the rats was apparently maintained throughout the study as there was no difference in plasma levels of AST and ALT between the control group and those that received the treatments (p>0.05). However, in a previous study, there was an increase in the levels of these enzymes in rats with induced diabetes and some treatments were beneficial for the reduction, especially of ALT[11].

Fructosamine dosage also did not differ from the control group to the other groups (p>0.05), although the rats in groups II and III had higher blood glucose levels than the control group on the twentieth day of the study (p<0.05). The evaluation of this parameter is important to verify glycated hemoglobin when plasma glucose levels remain elevated for some period[12] and is related to the complications of the disease. World Health Organization[10] states that people with impaired glucose tolerance and impaired fasting glucose are at high risk of

progressing to type 2 diabetes, so finding ways to control these parameters becomes important in prevention.

When the results of groups II and III were compared, no difference was observed (p>0.05). Thus, the treatment with glibenclamide was not influenced by the association with the extracts and was not efficient for the glycemic control of those animals that received STZ.

The result obtained was different from what was expected, as information from the literature shows that these compounds administered in isolation contribute to glycemic control.

We have some hypotheses to explain this fact. The first hypothesis refers to the dose of dry extracts and their concentrations in active phytocompounds. In Brazil, standardized extracts have been widely used in the production of personalized drugs in drug handling pharmacies through personalized medical prescription. Considering that 400 mg/kg of each extract were used and the information about these extracts provided by the manufacturers, that is, *A. membranaceus* dry extract standardized to 70%, *Peumus boldus* dry extract standardized to 0.5% of boldine and *Curcuma longa* dry extract standardized to 96% of curcuminoids, it can be inferred that the animals received about 280 mg of compounds from *A. membranaceus*, 2 mg of boldine and 384 mg of curcuminoids.

In fact, these doses differ from those found in the studies described in the literature. HUO et al.[13] studied the effect of aqueous extract of *A. membranaceus* in rats with induced diabetes at doses of 40 to 160 mg/kg and found a dose-dependent effect on blood glucose reduction. HERNÁNDEZ-SALINAS et al.[9] studied the effect of boldine 50mg/kg orally administered on the renal function of diabetic rats and concluded that the alterations found in diabetic rats were avoided, such as hyperglycemia, hypertension, and renal damage.

LAU et al.[14] in a review indicated that boldine controls oxidative stress and promotes endothelial protection at doses ranging up to 100 mg/kg/day. *Curcuma longa* was efficient when administered at a dose of 500 mg/kg for 4 weeks, reducing blood glucose, although it caused an increase in AST and ALT dosages[15].

The second hypothesis is the difference in treatment periods, so that prolonged treatment may show benefits, and in addition, it should also be considered that there may be differences between the extent of pancreatic injury by STZ. There are studies from 2 weeks to 6 months, on average[15-18].

The third hypothesis refers to the diet profile, as our animals received a balanced diet, with adequate nutritional composition and calories, while some authors evaluated the effectiveness during the consumption of a high-fat diet, evidencing the hyperglycemia and oxidative stress resulting from the disease[19]. We opted for the standard diet to carry out the treatment for the conditions closest to the recommended and, thus, to evaluate the response to treatment in this condition. We assume that the issues related to the diet being or not hypercaloric were the main factor that impacted the effectiveness of the treatment.

Under the conditions of this research, it is possible to state that the evaluated compounds were not effective in a type 2 diabetes condition in rats during 20 days of treatment and on the standard diet. Furthermore, the extracts did not interfere with the efficacy of glibenclamide, the data from groups II and III were similar. It is important to continue the investigation of these compounds in type 2 diabetes, varying all the conditions mentioned: treatment time, dose, extent of pancreatic damage and diet quality.

Diabetes remains a disease that deserves efforts on the part of the scientific community so that it is satisfactorily controlled and, if possible, even cured in the near future.

CONCLUSION

Daily oral administration of 400mg/kg of extracts of *A. membranaceus*, *Peumus boldus* and *Curcuma longa* for twenty days did not interfere with the efficacy of glibenclamide in a model of induced diabetes, and the values of fasting glucose, oral glucose tolerance and enzyme dosages liver diseases were equivalent between groups. These results cannot exclude extracts as a future treatment possibility, as adjustments can be made to optimize their actions on diabetes.

ACKNOWLEDGMENTS

We are grateful for the Scientific Initiation scholarship granted by the National Council for Scientific and Technological Development (CNPq).

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How to cite this article/Como citar este artigo:
Gonçalves MSG, Torrero TS. Effects of *Astragalus* membranaceus, *Peumus boldus* and *Curcuma longa* extracts in diabetic rats. J Health Biol Sci. 2022; 10(1):1-5.